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# Qbd Based RP-HPLC Method Development and Validation for Simultaneous Estimation of Amlodipine Besylate and Lisinopril Dihydrate in Bulk and Pharmaceutical Dosage Form

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The objective of this experiment was to develop and validate a simple, robust, and accurate QbD based Reverse-Phase High-Performance Liquid Chromatography method for Simultaneous estimation of Amlodipine besylate and Lisinopril dihydrate in bulk and Pharmaceutical Dosage form. A box-Behnken design was employed for optimizing the mobile phase, flow rate and pH of buffer, the optimized chromatographic conditions were Phosphate buffer: Methanol (25: 75 v/v), pH of buffer: 6.5 and flow rate: 1mL/min.

Furthermore formulation injected and observed that the additives do not interfere with the peak of Amlodipine besylate and Lisinopril dehydrate. Both drugs are well resolved and Retention times were found to be 2.332 min and 3.584 min respectively.

Linearity was observed in the concentration range of 10 µg to 50 µg/mL (r2=0.999). The accuracy range was 99.75 to 100.04%. Intra-day and Inter-day precision was found to be less than 2% RSD. The proposed method was useful for the best analysis of Amlodipine besylate and Lisinopril dihydrate in Bulk, pharmaceutical dosage forms and was successfully applied to routine analysis.

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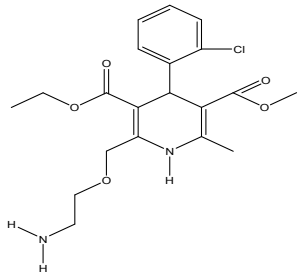
*Keywords: Amlodipine besylate; lisinopril dehydrate; RP-HPLC; Box-Behnken designs; QbD.*

## 1. INTRODUCTION

Amlodipine is a calcium channel blocker and a synthetic dihydropyridine having antihypertensive and antianginal effects [1-3]. Amlodipine prevents vascular and cardiac contraction by inhibiting the inflow of extracellular calcium ions into myocardial and peripheral vascular smooth muscle cells [4-6]. Amlodipine (AMD) is a 2-[(2-Aminoethoxy) methyl] compound. -4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid-3-ethyl-5-methyl ester Several spectroscopic methods, including RP-HPLC, HPTLC, LC-MS/MS, and LC-MS, have been published for estimating amlodipine alone and in combination with other medications [7-10]. Amlodipine is a medication that is used to treat hypertension and chronic stable angina. (LSNP), (S)-1-[N2-(1-Carboxy-3-phenylpropyl) - L-lysyl] is the chemical formula for lisinopril. -Dihydrate of L-proline It is a powerful, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for converting angiotensin I (ATl) to angiotensin II (Al) (ATII). ATII is a component of the renin-angiotensin-aldosterone system that regulates blood pressure (RAAS) [11-15]. Lisinopril is a medication that is used to treat hypertension and symptomatic congestive heart failure [16-21]. Several spectrophotometric methods for determining lisinopril in pharmaceutical tablets utilising various reagents have been published [22-26]. Methods for determining the first and second derivatives of spectrophotometric and spectrofluorometric data were devised. HPLC, micellar electro kinetic chromatography, and gas liquid chromatography have all been used to estimate the concentration of lisinopril alone and in combination with other medicines [27-31]. However, no strategy for combining AMD and LSN has been developed thus far. A successful attempt is made to estimate both medications at the same time. As a result, it was believed worthwhile to develop an accurate and fast RP-HPLC method for estimating AMD and LSN simultaneously from tablet formulations [32-35].

The objective of this experiment was to develop and validate a simple, robust, and accurate QbD based Reverse-Phase High-Performance Liquid Chromatography method for Simultaneous estimation of Amlodipine besylate and Lisinopril dihydrate in bulk and Pharmaceutical Dosage form.

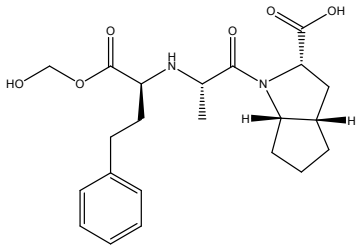
**Structure:**



**Structure of Amlodipine Besylate**

**IUPAC Name**: 3-Ethyl 5-methyl -2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzenesulfonate

**Structure:**



**Structure of Lisinopril**

**IUPAC Name**: (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Cipla Pharmaceutical Ltd, India, sent a gift sample of lisinopril (LSN) and amlodipine (AMD) reference standards. AMLOPRESS-L (Cipla), a pharmaceutical preparation containing 5 mg of lisinopril and 5 mg of amlodipine comparable to amlodipine besylate, was acquired from a local pharmacy store. Merck Lie Sciences Pvt. Ltd., Mumbai, supplied HPLC grade methanol.

### 2.2 Instrumentation

The proposed work was carried out on Isocratic HPLC (Shimadzu) with LC20AD, PU2080 pump,

UV 2075 plus detector, and Phenomenex C18 column (5 µm particle size) was used. The software used was Borwin.

## 2.3 Methods

### 2.3.1 Preliminary analysis of drug

The colour and texture of Amlodipine besylate (AMB) and Lisinopril Dihydrates (LSD) were matched to known drug bank features. Amlodipine is slightly soluble in water and only slightly soluble in ethanol, whereas lisinopril dehydrates is little soluble in water, only slightly soluble in methanol, and almost completely insoluble in ethanol. The solutions were subjected to UV examination by scanning them at 200-400 nm.

**For Assay Preparation**

### 2.3.2 Chromatographic condition

Shimadzu series LC 2010 A chromatographic system was used for the analysis (pump Quaternary system). Separation was performed on a Kromasil C8 (4.6mm x 250mm, 5 particle size) column at 30°C, with a flow rate of 1.00mL per min. and an isocratic mobile phase composed of Buffer & Acetonitrile in a 60:40 ratio. Orthophosphoric acid was used to raise the pH to 3.6. The concentrations of lisinopril and amlodipine were determined using a UV detection method at 215nm, with an injection volume of 20L and a run period of 7 minutes.

### 2.3.3 Selection of detection wavelength

Further dilutions of the standard stock solution were made with water and scanned over the range of 200-400 nm, with the spectra being overlain. Amlodipine and lisinopril were found to have significant absorbance at 215 nm.

### 2.3.4 Preparation mobile phase

90 ml of HPLC grade Methanol was mixed with 10 ml of water in a 90:10 v/v ratio. Trimethylamine and orthophosphoric acid were used to modify the pH to 4.5, 5.5, and 6.5. The solution was filtered through a 0.45 membrane filter and then sonicated for 10 minutes in a sonicator bath.

### 2.3.5 Preparation of standard solution

Weigh correctly 50 mg of Lisinopril and 50 mg of Amlodipine besylate, transfer to a 100 mL volumetric flask, dissolve in 70 mL of mobile phase, and build volume up to the mark with mobile phase to obtain a stock solution containing 500g/ml of Lisinopril and 500g/ml of Amlodipine besylate. The final solution was prepared by pouring 5 mL of this solution into a 100 mL volumetric flask and filling it with mobile phase to obtain 50g/mL of Lisinopril and 50g/mL of Amlodipine besylate, respectively. Figure 1 depicts a typical chromatogram of conventional Lisinopril and Amlodipine.

### 2.3.6 Preparation of sample solution

For the assay, 20 tablets of Lisinopril labelled as having 5mg and 5 mg of Amlodipine besylate, together with excipients, were precisely weighed and ground into a fine powder. Take an accurate weight of powder equivalent to 5 mg of lisinopril and 5 mg of amlodipine and transfer to a 100 ml volumetric flask, then add 50 ml of mobile phase and sonicate for 10 minutes. Cool it down and increase the volume with mobile phase. Filter a portion of this solution using a 0.45m membrane syringe filter. The final solution was made by putting 5 ml of this filtered solution into a 100 ml volumetric flask and increasing the volume by adding mobile phase to obtain 50g/ml of Lisinopril and 50g/ml of Amlodipine besylate, respectively. Figure 2.0 depicts a typical chromatogram of the samples Lisinopril and Amlodipine.

For Content uniformity, one tablet was placed in to each of ten 100 ml volumetric flask. Approximately 70 ml of mobile phase was added to each volumetric flask &sonicate till tablets were dispersed in the solution. Cool the resultant solutions and make volume up to the mark with the mobile phase. Shake the solution well for uniform distribution. Filtered a portion of solution by using 0.45µm membrane syringe filter & then filtrate was injected for analysis.

A figure 1 & 2 represents the typical sample chromatogram of Lisinopril and Amlodipine respectively.

## 2.4 Design of Experiment

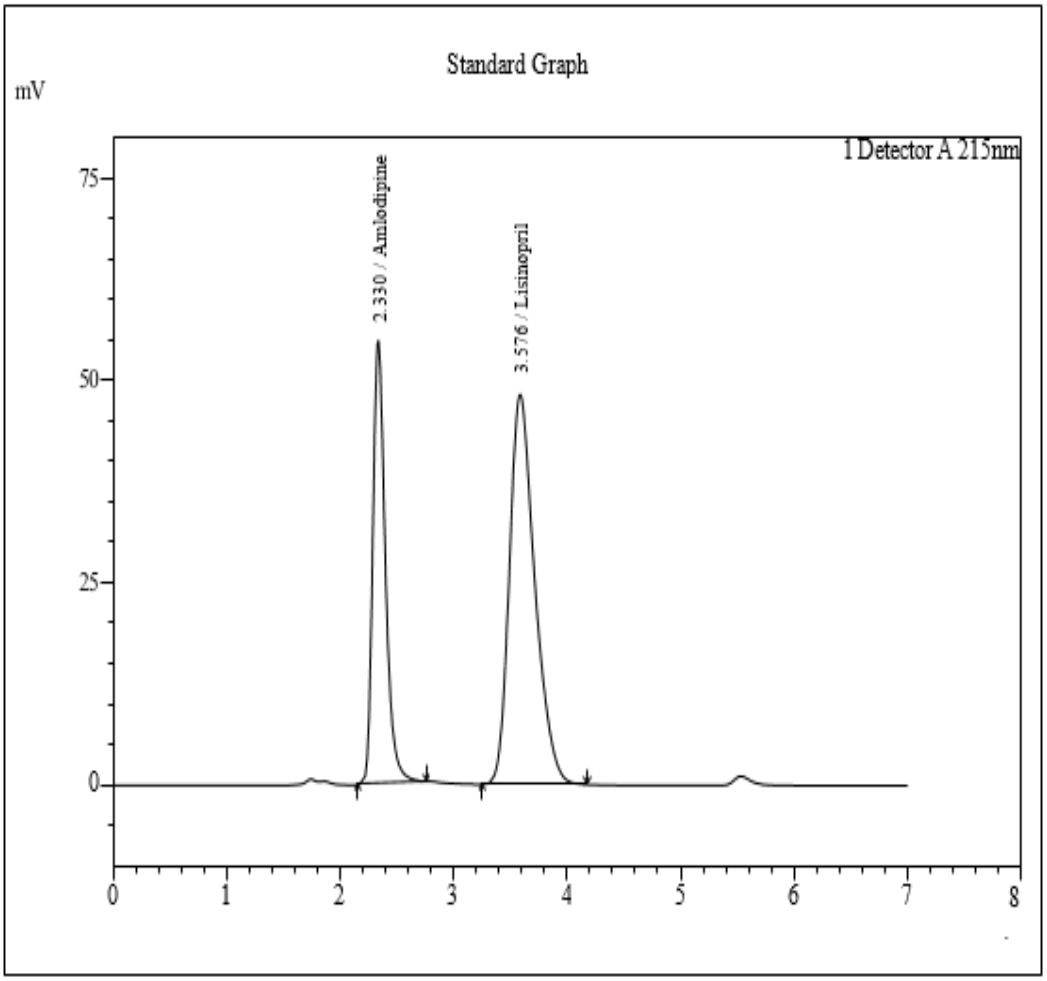
Box-Behnken designs are response surface designs that are specifically designed to require only three levels, denoted as -1, 0 and +1. Box-Behnken designs are offered for three to twenty-one factors. They are created by merging two-level factorial and incomplete block designs. This approach generates designs with desirable statistical features while also requiring a fraction

of the experiments required for a three-level factorial. The quadratic model is adequate because there are just three layers. For the majority of these designs, blocking choices are also available. This design may also include categorical factors. The number of runs generated will be doubled by the number of categorical factor level combinations.

Dependent factors were selected as mobile phase, pH of aqueous phase and flow rate and Independent factors were selected as retention time, peak area, theoretical plates and peak asymmetry. The C18 column is used for proposed method.

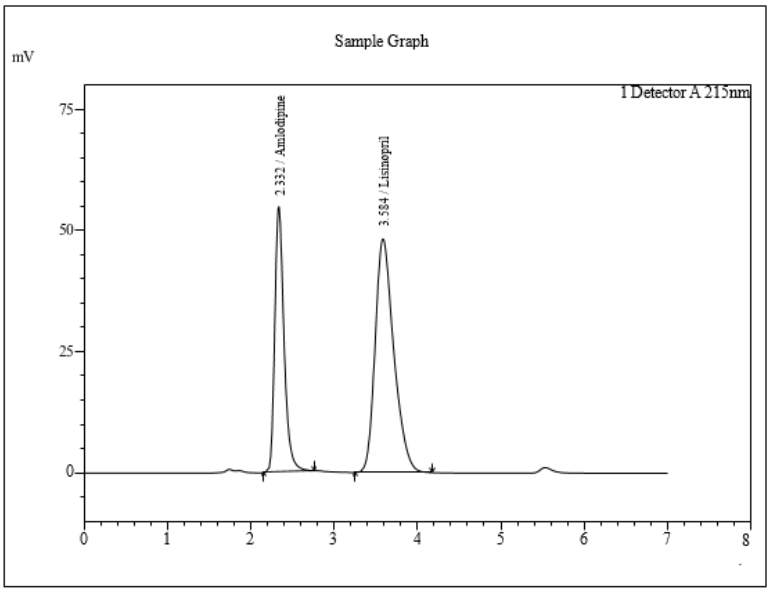
**2.4.1 Following mobile phases selected**

- ✓ Phosphate buffer: Methanol
- ✓ Water: Methanol
- ✓ Water : Acetonitrile




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.330 | Amlodipine | 422610 | 36.102 | 1.021 | 9924 |
| 2 | 3.576 | Lisinopril | 747963 | 63.898 | 1.132 | 12027 |
| Total | | | 1170573 | 100.000 | | |

**Fig. 1. Standard preparation Chromatogram**




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.332 | Amlodipine | 422752 | 36.097 | 1.024 | 9903 |
| 2 | 3.584 | Lisinopril | 748393 | 63.903 | 1.137 | 12035 |
| Total | | | 1171145 | 100.000 | | |

**Fig. 2. Chromatogram of sample preparation**

### 2.4.2 Box-Behnken design facilitate only one solvent of mobile phase at a time

- ✓ Change Mobile phase proportion Range: 75-95% (Consider Organic Phase)
- ✓ Change pH Range: 4.00 to 6.00 mmol/L
- ✓ Flow rate: 0.9 to 1.1 mL/min

The Box-Behnken design produced 12 runs (Table 1) with varying pH, mobile phase percentage, and flow rate. For each mobile phase, the same method was followed. The total number of runs during the three mobile stages was 36. By maximising desired parameters and decreasing undesired ones, optimization involves finding an alternative with the most cost effective or greatest feasible performance under the given restrictions. Maximization, on the other hand, involves attempting to achieve the highest or maximum result or outcome without regard for cost or expense.

**Table 1. Trails of box-behnken design**

| Sr. No | Mobile Phase Composition (Organic Phase, v/v) | pH of Buffer mmol/L | Flow Rate (mL/min) |
|---|---|---|---|
| 1 | 95.00 | 5.50 | 1.10 |
| 2 | 85.00 | 4.50 | 1.10 |
| 3 | 85.00 | 6.50 | 1.10 |
| 4 | 95.00 | 4.50 | 1.00 |
| 5 | 75.00 | 5.50 | 1.10 |
| 6 | 85.00 | 4.50 | 0.90 |
| 7 | 75.00 | 6.50 | 1.00 |
| 8 | 95.00 | 5.50 | 0.90 |
| 9 | 75.00 | 4.50 | 1.00 |
| 10 | 85.00 | 6.50 | 0.90 |
| 11 | 75.00 | 5.50 | 0.90 |
| 12 | 95.00 | 6.50 | 1.00 |

## 3. RESULTS AND DISCUSSION

### 3.1 Optimization Result

#### 3.1.1 Screening design for suitable chromatographic condition

Determination of chromatographic condition is based on peak parameters of both drugs.

After taking runs on HPLC, we got following results of different mobile phase with different pH and different flow rate. To have better understanding the peak properties used remarks like Extremely Satisfactory, Satisfactory, More Satisfactory, partially Satisfactory and Dissatisfactory.

**The following tables show the results of numerous trials with organic phase compositions of 75 percent v/v.**

**Table 2. Runs performed at mobile phase (75:25 v/v) with aqueous phase pH 6.5.**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Peak qualities that are good, a shorter retention period with more theoretical plates, and a lower asymmetry factor | Extremely Satisfactory |
| 2 | Water: Methanol | Lower theoretical plates and less peak height | Satisfactory |
| 3 | Water: Acetonitrile | Only one peak appeared (Amlodipine) another peak is very small (Lisinopril) | Dissatisfactory |

**Table 3. Runs performed at mobile phase (75:25 v/v) with aqueous phase pH 5.5.**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Less peak asymmetry but less theoretical plates | Satisfied |
| 2 | Water: Methanol | Greater peak Asymmetry and lower theoretical plates | Partially satisfactory |
| 3 | Water: Acetonitrile | Resolution of Peaks is not good | Very Dissatisfactory |

**Table 4. Runs performed at mobile phase (75:25 v/v) with aqueous phase pH 4.5**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Less peak asymmetry with more theoretical plates and good retention time | Partly Satisfactory |
| 2 | Water: Methanol | Good Peak Properties but Resolution is not Good | Partly Satisfactory |
| 3 | Water: Acetonitrile | The peak of lisinopril not appeared | Dissatisfactory |

**Results of various trials, having organic phase composition 85 % v/v are shown in following tables.**

**Table 5. Runs performed at mobile phase (85:15 v/v) with aqueous phase pH 6.5**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Less theoretical plates | Satisfied |
| 2 | Water: Methanol | Broad Peak Appeared | Partially satisfactory |
| 3 | Water: Acetonitrile | Broad Peak Appeared and noise exist | Very Dissatisfactory |

**Table 6. Runs performed at mobile phase (85:15 v/v) with aqueous phase pH 4.5**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Two peaks appeared | Dissatisfactory |
| 2 | Water: Methanol | Asymmetric factor is more | Not Satisfactory |
| 3 | Water: Acetonitrile | No Peak found | Very Dissatisfactory |

**The following tables show the results of numerous trials with organic phase compositions of 95 percent v/v.**

**Table 7. Runs performed at mobile phase (95:05 v/v) with aqueous phase pH 6.5**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Broad Peak appeared | Not Satisfactory |
| 2 | Water : Methanol | No Peak found | Not Satisfactory |
| 3 | Water: Acetonitrile | No Peak found | Very Dissatisfactory |

**Table 8. Runs performed at mobile phase (95:05 v/v) with aqueous phase pH 5.5**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Greater Peak Asymmetry | Not Satisfactory |
| 2 | Water : Methanol | Greater Peak Asymmetry | Not satisfactory |
| 3 | Water: Acetonitrile | Greater Peak Asymmetry | Not satisfactory |

**Table 9. Runs performed at mobile phase (95:05 v/v) with aqueous phase pH 4.5.**

| Sr. no. | Composition | Observation | Remarks |
|---|---|---|---|
| 1 | Phosphate buffer: Methanol | Lower retention time | Not satisfactory |
| 2 | Water : Methanol | Lower theoretical plates | Not satisfactory |
| 3 | Water: Acetonitrile | Lower theoretical plates | Not satisfactory |

**Table 10. Trials performed on C18 column at mobile phase (80:20 v/v) with aqueous phase pH 6 are extremely Satisfactory. Design expert has optimized the following chromatographic conditions with respect to desirability value**

| Sr. No | Mobile Phase Composition (Organic Phase, v/v) | pH of Buffer mmol/L | Flow Rate (mL/min) | Retention Time | Asymmetry | Theoretical Plates |
|---|---|---|---|---|---|---|
| **Amlodipine besylate** | | | | | | |
| 1 | 95.00 | 5.50 | 1.10 | 0.91 | 2.137 | 9902 |
| 2 | 85.00 | 4.50 | 1.10 | 1.04 | 2.143 | 8364 |
| 3 | 85.00 | 6.50 | 1.10 | 0.952 | 1.988 | 8514 |
| 4 | 95.00 | 4.50 | 1.00 | 0.121 | 1.997 | 10001 |
| 5 | 75.00 | 5.50 | 1.10 | 2.401 | 1.328 | 9237 |
| 6 | 85.00 | 4.50 | 0.90 | 0.987 | 2.223 | 7986 |
| 7 | 75.00 | 6.50 | 1.00 | 2.332 | 1.105 | 9034 |
| 8 | 95.00 | 5.50 | 0.90 | 0.321 | 1.549 | 11794 |
| 9 | 75.00 | 4.50 | 1.00 | 2.458 | 1.101 | 9464 |
| 10 | 85.00 | 6.50 | 0.90 | 0.889 | 1.643 | 8787 |
| 11 | 75.00 | 5.50 | 0.90 | 2.547 | 1.212 | 9912 |
| 12 | 95.00 | 6.50 | 1.00 | 0.221 | 1.697 | 11014 |
| **Lisinopril Dihydrates** | | | | | | |
| 1 | 95.00 | 5.50 | 1.10 | 0.997 | 1.592 | 12547 |
| 2 | 85.00 | 4.50 | 1.10 | 1.871 | 1.986 | 10985 |
| 3 | 85.00 | 6.50 | 1.10 | 1.627 | 1.414 | 11987 |

| Sr. No | Mobile Phase Composition (Organic Phase, v/v) | pH of Buffer mmol/L | Flow Rate (mL/min) | Retention Time | Asymmetry | Theoretical Plates |
|---|---|---|---|---|---|---|
| 4 | 95.00 | 4.50 | 1.00 | 1.223 | 2.234 | 12996 |
| 5 | 75.00 | 5.50 | 1.10 | 3.687 | 1.637 | 15759 |
| 6 | 85.00 | 4.50 | 0.90 | 1.749 | 1.913 | 14967 |
| 7 | 75.00 | 6.50 | 1.00 | 3.584 | 1.264 | 10211 |
| 8 | 95.00 | 5.50 | 0.90 | 1.139 | 1.567 | 110487 |
| 9 | 75.00 | 4.50 | 1.00 | 3.741 | 2.031 | 10352 |
| 10 | 85.00 | 6.50 | 0.90 | 1.6 | 1.521 | 11252 |
| 11 | 75.00 | 5.50 | 0.90 | 3.629 | 1.694 | 12065 |
| 12 | 95.00 | 6.50 | 1.00 | 0.991 | 1.497 | 11988 |

**Table 11. Optimized trials suggested by software based on desirability value**

| Sr. no. | Amount of Methanol | pH of buffer | Flow rate | Retention time | Tailing factor | Theoretical plates | Desirability |
|---|---|---|---|---|---|---|---|
| **Amlodipine besylate** | | | | | | | |
| **1** | 75.00 | 6.50 | 1.02 | 2.28942 | 1.2396 | 9006.65 | 0.893 |
| **Lisinopril Dihydrates** | | | | | | | |
| 1 | 75.00 | 6.50 | 1.02 | 3.43954 | 1.35292 | 10219.6 | 0.893 |

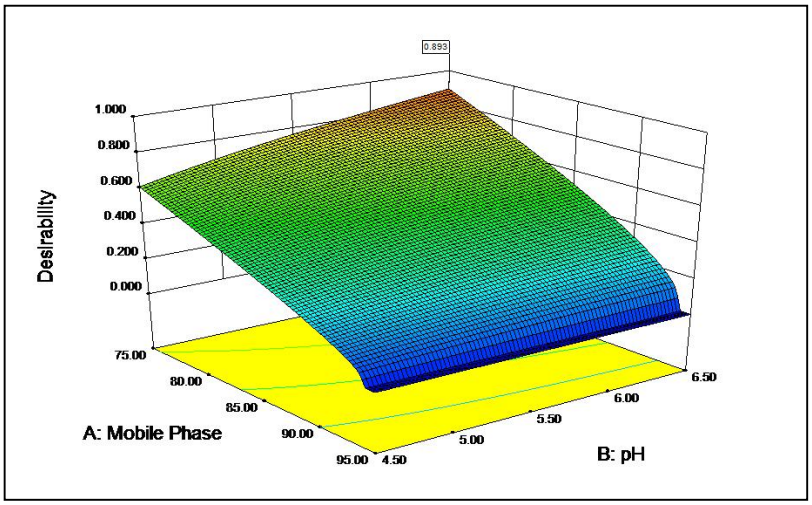



**Fig. 3. 3D Diagram of Desirability Value**

This process begins by creating a desirability function for each individual response. The scale of the individual desirability function spans from i=0 (totally unwanted reaction) to I =1 (entirely desired answer). The experiment was chosen based on the highest attractiveness value. As a result, the first experiment with desirability one (i=1) was chosen for method optimization.

### 3.1.2 Optimized chromatographic conditions

Mobile phase: Phosphate buffer: Methanol (25: 75 v/v), pH of buffer: 6.5, Analytical column: $C_{18}$ column Waters XBridge (4.6× 250mm id. particle size 5µm), UV detection: 215nm, Injection volume: 10 µL, Flow rate: 1.00 mL $min^{-1}$, Temperature: Ambient, Run time: 10 min

### 3.1.3 Effect of independent variables on retention time (X):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of AMB-74.67 & LSD-104.40, *p* value less than 0.005 and $R^2$ value of AMB-0.9655 & LSD-0.9126. There is only a (AMB & LSD) 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of % C.V. and adjusted $R^2$ were 18.05 & 16.54 and 0.9526 & 0.9038 respectively. The model for response X (Retention time) is as follows:

The equation for response surface quadratic model is as follows

**Retention Time (Amlodipine) = +11.41577 - 0.11344 * Mobile Phase -0.026500 * pH- 0.43875 * Flow Rate**

**3.1.4Retention Time (Lisinopril) = +13.08735 - 0.12864 * Mobile Phase**

Fig.4 shows a graphical representation of pH of buffer (B) and amount of Methanol (A), while flow rate (C) is maintained constant at its optimum of 1.02 mL $min^{-1}$. Change in pH of buffer showed slightly change in retention time (X), also increase in amount of Methanol showed decreases the retention time.

**Fit summary:** Linear model was suggested by the software.

ANOVA: ANOVA of developed full three level factorial models for retention time ($Y_1$).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms.

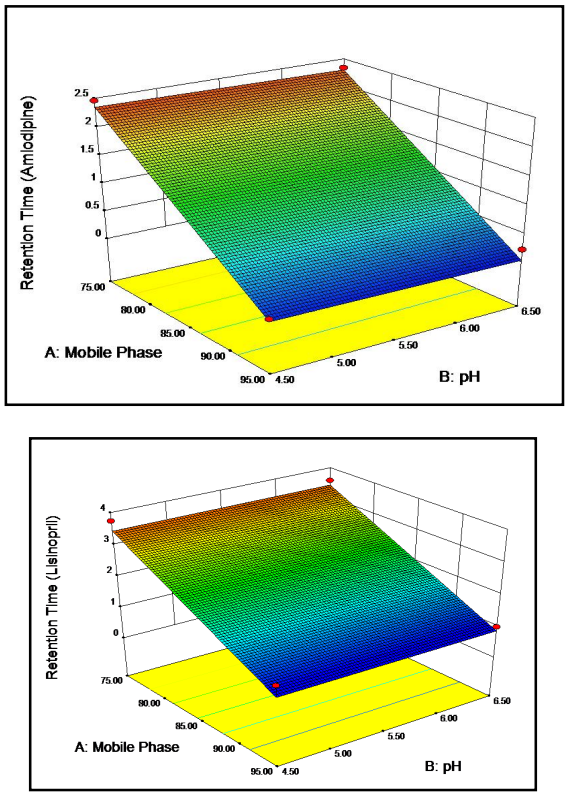



**Fig. 4. Three-dimensional plot for retention time as a function of pH of buffer and amount of Methanol, Constant factor (flow rate- 1.02 mL $min^{-1}$)**

**Table 12. Significance of *p* value on model terms of retention time**

| Model terms | p value (AMB) | Effect of factor (AMB) | p value (LSD) | Effect of factor (LSD) | Remarks |
|---|---|---|---|---|---|
| A | 0.0001 | 10.29 | 0.0001 | 13.24 | Significant |
| B | 0.7359 | 5.618E-003 | - | - | Insignificant |
| C | 0.5790 | 0.015 | - | - | Insignificant |
| **Overall model** | **0.0001** | | **0.0001** | | **Significant** |

### 3.1.5Effect of independent variables on tailing factor (Y)

Following the application of the experimental design, the proposed Response Surface Linear Model was determined to be significant, with model F values of AMB-3.62 & LSD-15.86, p value less than 0.005, and R2 values of AMB-0.5758 & LSD-0.8561. AMB-6.47 percent and LSD-0.10 percent of the time, a "Model F-Value" this significant could arise owing to noise. The percent C.V. values were AMB-19.13 & LSD-7.50, while the adjusted R2 was AMB-0.4167 & LSD-0.8021 correspondingly. The model for response

Asymmetric Factor (Amlodipine) = -1.62415 +0.032925 * Mobile Phase - 0.12888 * pH+1.21125 * Flow Rate

Asymmetric Factor (Lisinopril) = +3.19458 +3.30000E-003 * Mobile Phase -0.30850 * pH - 0.082500 * Flow Rate

Fig.5 depicts a graphical representation of the pH of the buffer (B) and the amount of ACN (A), with the flow rate (C) held constant at its optimum of 1.02 mL min-1. A drop in buffer pH decreases the tailing factor, which has a synergistic effect on response (Y), however increasing the amount of Methanol had no significant influence on the asymmetry.

**Fit summary:** Response Surface Linear Model was suggested by the software.

ANOVA: ANOVA of developed CCD model for tailing factor ($Y_2$).

Model terms are important when the "Prob > F" (p- value) is less than 0.0500. In this scenario, B denotes important model terms.

### 3.1.6Effect of independent variables on theoretical plates (Z)

Following the application of the experimental design, the proposed Response Surface Linear Model was determined to be significant, with model F values of AMB-1.23 & LSD-1.09, p value less than 0.005, and R2 values of AMB-0.3156 & LSD-0.5657. AMB-36.06 & LSD-47.40 percent of the time, a "Model F-Value" this significant could arise owing to noise. The percent C.V. values were AMB-11.31 & LSD-135.63, while the adjusted R2 was AMB-0.0590 & LSD-0.0446. The response Z (theoretical plates) model.

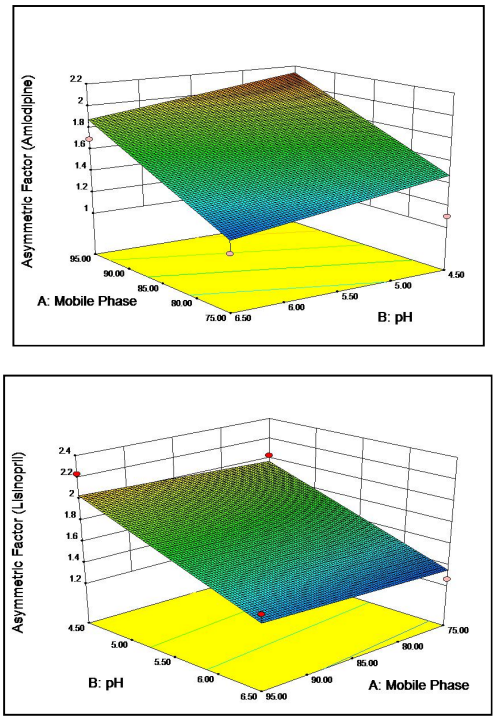



**Fig. 5. Tailing factor plotted in three dimensions as a function of buffer pH and Methanol concentration (flow rate- 1.02 mL min-1)**

**Table 13. Significance of *p* value on model terms of tailing factor**

| Model terms | p value (AMB) | Effect of factor (AMB) | p value (LSD) | Effect of factor (LSD) | Remarks |
|---|---|---|---|---|---|
| A | 0.0198 | 0.87 | 0.4842 | 8.712E-003 | **Significant** |
| B | 0.2888 | 0.13 | 0.0001 | 0.76 | Insignificant |
| C | 0.3167 | 0.12 | 0.8591 | 5.445E-004 | Insignificant |
| Overall model | 0.0647 | Insignificant | 0.0010 | | Significant |

Theoretical Plates (Amlodipine) =+6143.12500+63.30000 * Mobile Phase+191.75000 * pH - 3077.50000 * Flow Rate

Theoretical Plates (Lisinopril)=-2.06587E + 006+26773.10000 * Mobile Phase-10432.875 * pH +1.97300E+006 * Flow Rate -21.67500 * Mobile Phase * pH -25408.50000 * Mobile Phase * Flow Rate +11792.50000 * pH * Flow Rate

Fig.6 depicts a graphical representation of the amount of Acetonitrile (A) and the pH of the buffer (B), while the flow rate (C) is held constant at its optimum value of 1.02 mL min-1. A drop in buffer pH had no influence on the number of theoretical plates (Z), however increasing the amount of Acetonitrile increased the response.

**Fit summary:** Linear model was suggested by the software

ANOVA: ANOVA of developed CCD model for theoretical plates ($Y_3$).

Model terms are important when the "Prob > F" (p- value) is less than 0.0500. In this scenario, A value is significant in terms of model terms.

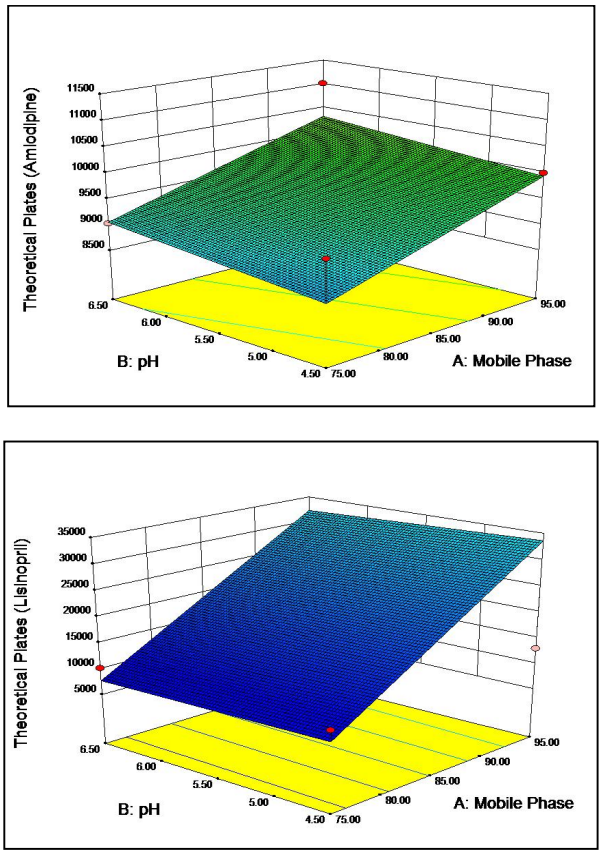



**Fig. 6. Three-dimensional plot for theoretical plates as a function of pH of buffer and amount of Methanol, Constant factor (flow rate- 1.02 mL min$^{-1}$)**

**Table 14. Significance of *p* value on model terms of theoretical plates**

| Model terms | p value (AMB) | Effect of factor (AMB) | p value (LSD) | Effect of factor (LSD) | Remarks |
|---|---|---|---|---|---|
| A | 0.1341 | 3.206E+006 | 0.2603 | 1.241E+009 | Insignificant |
| B | 0.6272 | 2.941E+005 | 0.9627 | 1.864E+006 | Insignificant |
| C | 0.4412 | 7.577E+005 | 0.2694 | 1.188E+009 | Insignificant |
| Overall model | 0.3606 | - | 0.4740 | - | Insignificant |

**Calibration curves:** Pipette out suitable aliquots from each standard stock solution into a series of 10 ml volumetric flasks for each medication. The capacity was filled to the mark with mobile phase to produce a set of solutions with concentrations ranging from 10, 20, 30, 40, and 50 g/ml for each medication. Separate triplicate dilutions of each medication concentration were made. From these duplicate solutions, 10 l injections of each drug concentration were injected separately into the RP-HPLC apparatus and chromatographed under the conditions stated above. Both medications were evaluated using a UV detector set to 215nm. Peak areas were measured for each peak and plotted against concentrations to create the standard calibration curves.

## 4. ANALYSIS OF THE MARKETED FORMULATION

Twenty tablets were weighed and finely ground into powder. The tablet powder containing 5 mg of amlodipine and 5 mg of lisinopril was transferred to a 100 ml volumetric flask and dissolved in mobile phase for 30 minutes in an ultra sonicator. Finally, mobile phase was used to bring the volume up to the required level. The solution was passed through a 0.45 m membrane filter paper before being filtered. This solution was diluted further with mobile phase, and a standard stock solution of AMD was added to produce a mixed sample solution comprising 5 mg amlodipine and 5 mg lisinopril.

Under the chromatographic conditions mentioned above, a total of 20 l of sample solution was injected into the sample injector five times. At 215 nm, the area of each peak was measured. The peak area of AMD and LSN was used to calculate the amount of each drug present in the sample (n = 5). A typical chromatogram of AMD and LSN in tablet formulation (Fig.1).

## 4.1 Method Validation

The proposed RP-HPLC method was validated as per ICH guidelines.

### 4.1.1 Linearity

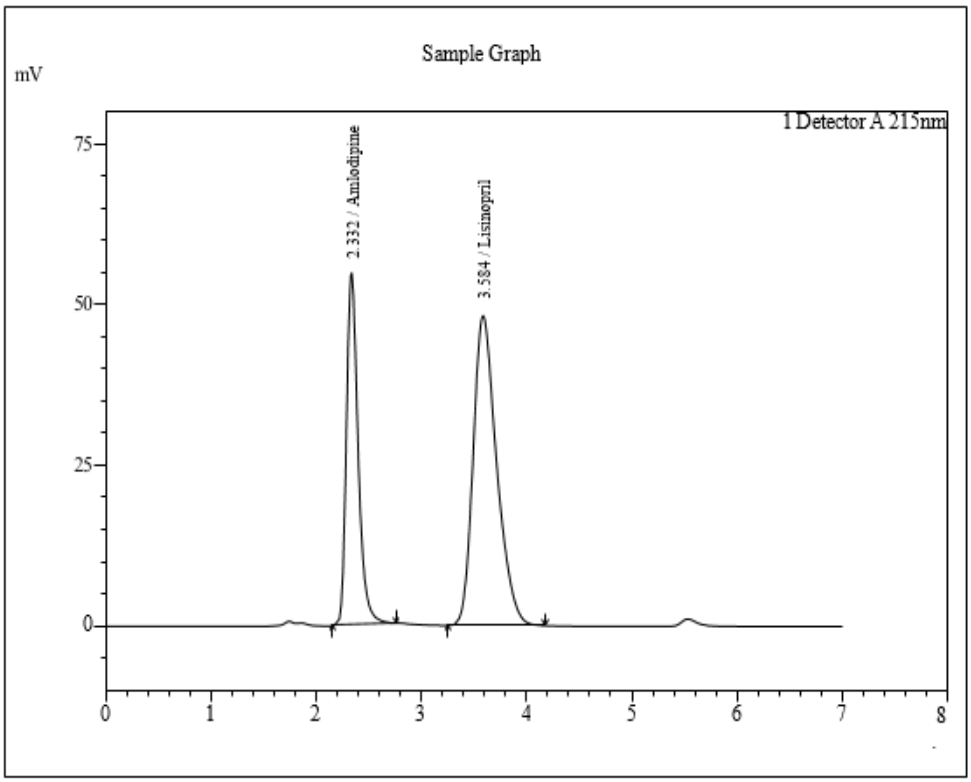
Several aliquots of standard AML and LIS solutions were placed in various 10 ml volumetric flasks and the capacity was filled with mobile phase to achieve a final concentration of AML and LIS of 10-50 g/ml, respectively. The UV-Vis detector at 215 nm was used for the evaluation, and the peak area for each peak was recorded. The calibration curve was drawn as a plot of concentration versus peak area. The calibration curve slope and intercept values were y = 5E-05x - 0.1239 (R2 = 0.9996) for AML and y = 3E-05x - 0.1259 (R2 = 0.9999) for LIS (Fig. 8 & 9).

### 4.1.2 Specificity

The RP-HPLC method's specificity was determined by comparing the chromatograms of mixed standards and sample solutions. Retention time (t R), resolution (R S), and tailing factor (T f) were all computed. There was a strong association between the results of mixed standards and sample solutions as shown in Table. 16.

**Table 15. Linearity data**

| Sr.No. | Injection Volume | Concentration (µg/ml) | Peak Area | |
|---|---|---|---|---|
| | | | Amlodipine | Lisinopril |
| 1 | 1 | 10 | 211826 | 374397 |
| 2 | 2 | 20 | 422752 | 748393 |
| 3 | 3 | 30 | 636158 | 1124590 |
| 4 | 4 | 40 | 855604 | 1498886 |
| 5 | 5 | 50 | 1049180 | 1859983 |




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.332 | Amlodipine | 422752 | 36.097 | 1.024 | 9903 |
| 2 | 3.584 | Lisinopril | 748393 | 63.903 | 1.137 | 12035 |
| Total | | | 1171145 | 100.000 | | |

**Fig. 7. Typical chromatogram of amlodipine (AMD) RT (2.332 min.) and Lisinopril (LSN) RT (3.584 min.)**

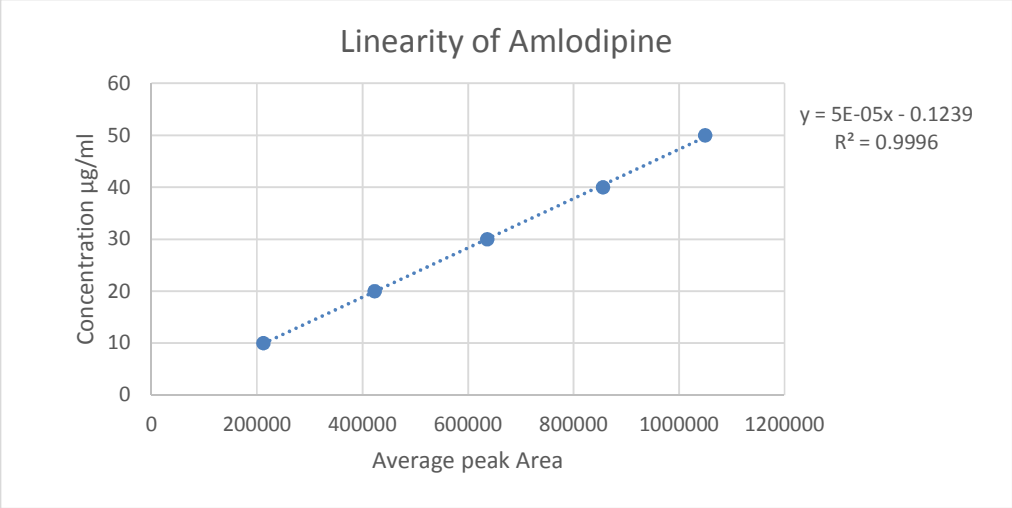



**Fig. 8. Calibration curve of amlodipine besylate**

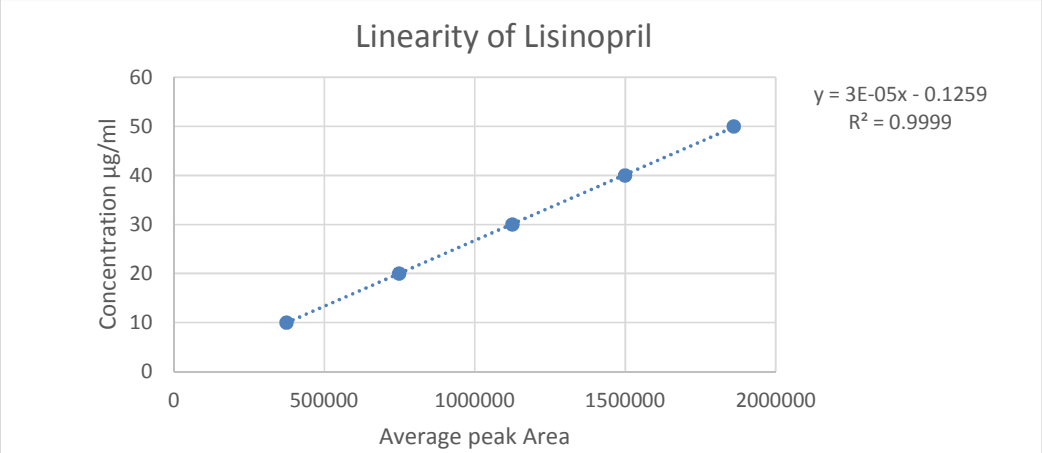
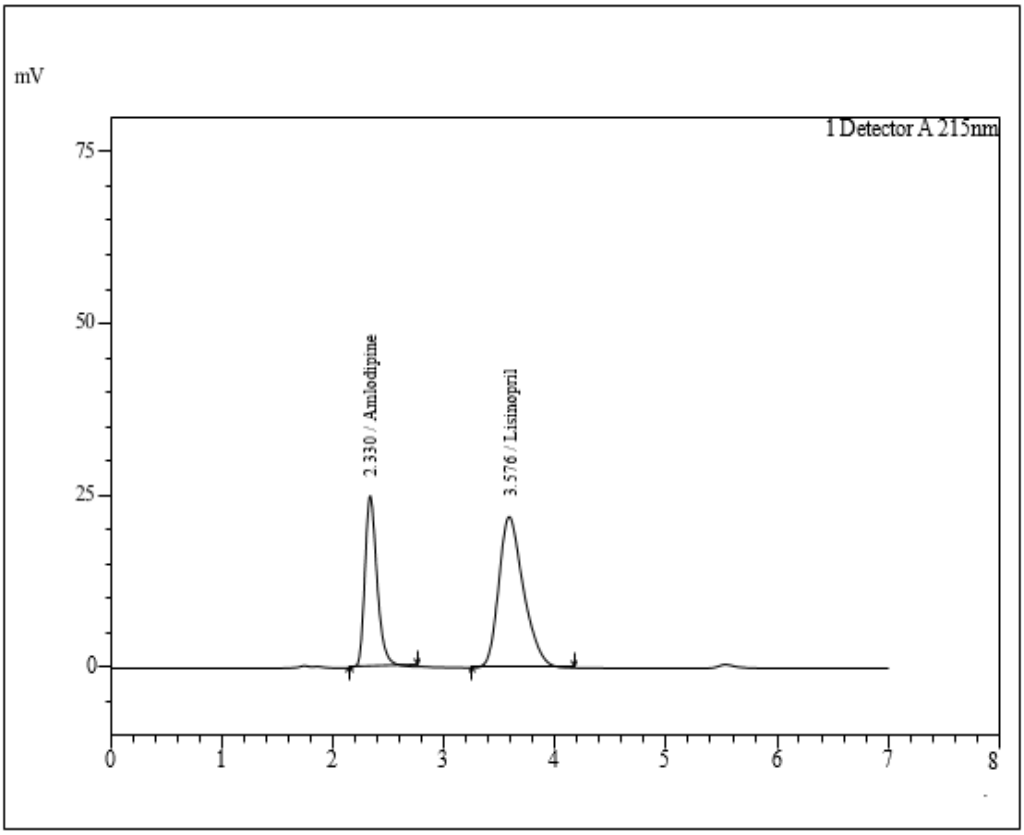
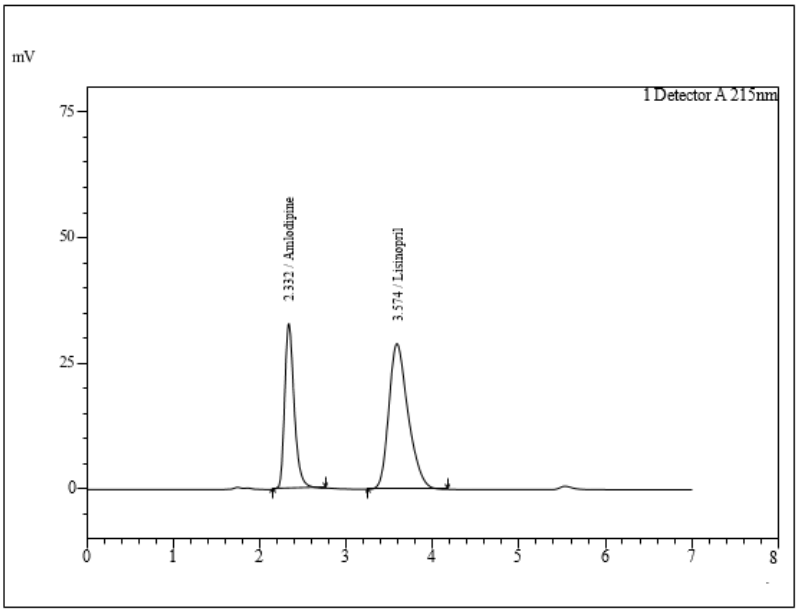



**Fig. 9. Calibration curve of lisinopril dehydrate**



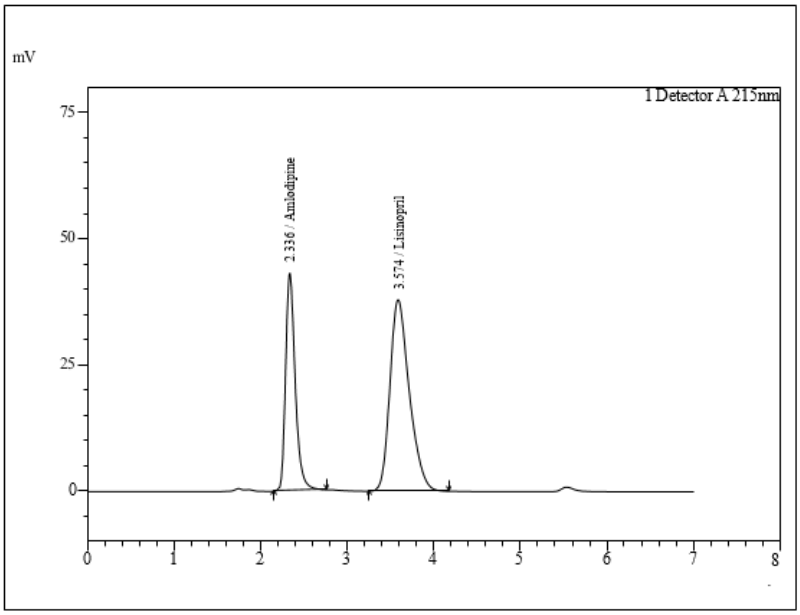

| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.330 | Amlodipine | 211826 | 36.134 | 1.014 | 10021 |
| 2 | 3.576 | Lisinopril | 374397 | 63.866 | 1.130 | 12023 |
| Total | | | 586223 | 100.000 | | |

**Fig. 10. Chromatogram of injection 1**



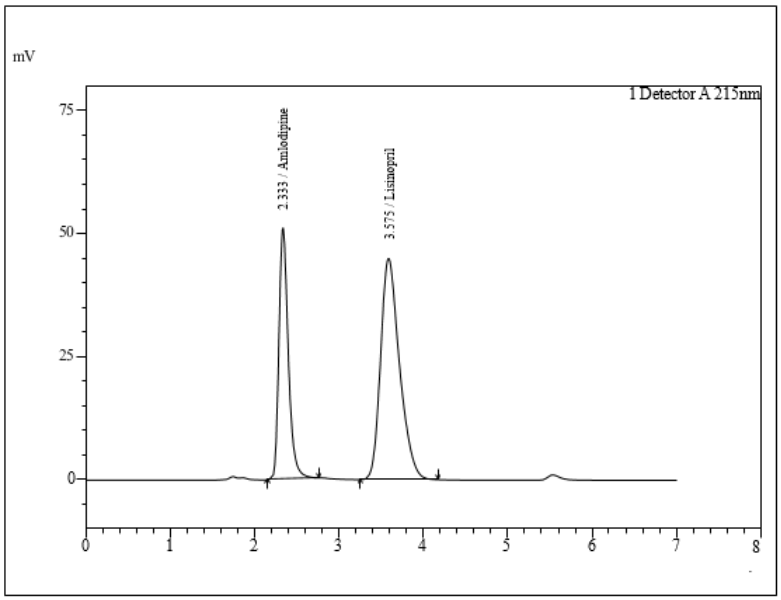

| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.332 | Amlodipine | 422752 | 36.134 | 1.014 | 10021 |
| 2 | 3.574 | Lisinopril | 748393 | 63.866 | 1.130 | 12023 |
| Total | | | 1171145 | 100.000 | | |

**Fig. 11. Chromatogram of injection 2**



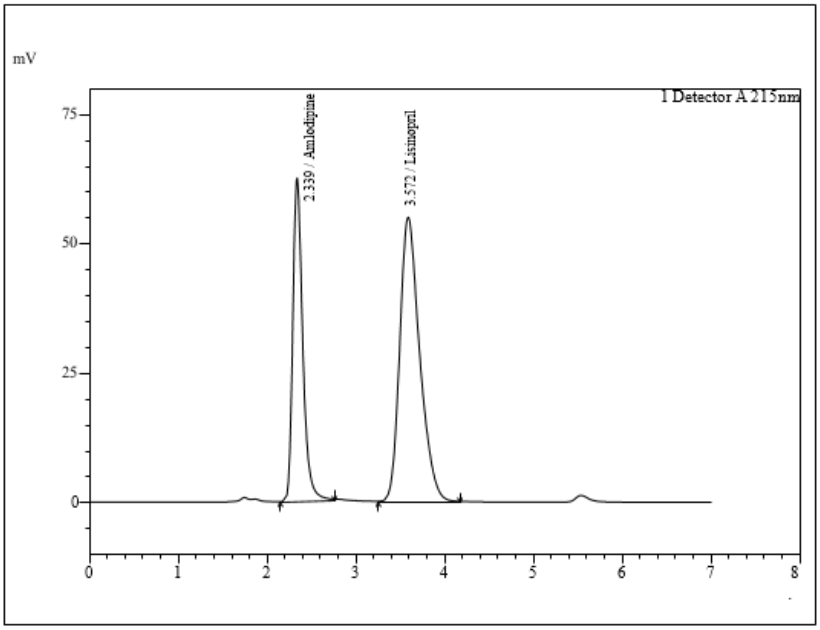

| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.336 | Amlodipine | 636158 | 36.129 | 1.012 | 10025 |
| 2 | 3.574 | Lisinopril | 1124590 | 63.871 | 1.132 | 12023 |
| Total | | | 1760748 | 100.000 | | |

**Fig. 12. Chromatogram of injection 3**




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.333 | Amlodipine | 855604 | 36.339 | 1.011 | 10035 |
| 2 | 3.575 | Lisinopril | 1498886 | 63.660 | 1.132 | 12014 |
| Total | | | 2354490 | 100.000 | | |

**Fig. 13. Chromatogram of injection 4**




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.339 | Amlodipine | 1049180 | 35.878 | 1.012 | 10005 |
| 2 | 3.572 | Lisinopril | 1859983 | 64.121 | 1.123 | 12096 |
| Total | | | 2909163 | 100.000 | | |

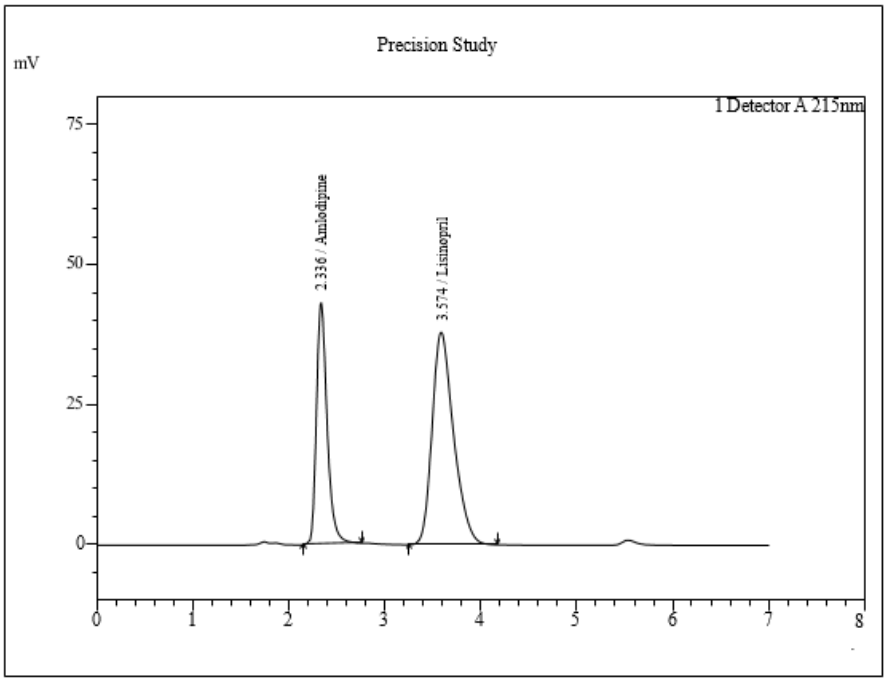
**Fig. 14. Chromatogram of injection 5**

**Table 16. Specificity**

| Concentration | API Area | Tablet Area |
|---|---|---|
| 20 | 6158789 | 6158789 |
| 20 | 6342365 | 6098869 |
| 20 | 6242599 | 6024669 |
| 20 | 6205322 | 6128992 |
| 20 | 6190789 | 6032849 |
| 20 | 6140789 | 5959863 |
| Mean | 6213442 | 6067339 |
| **SD** | 72547.60 | 74457.66 |
| **RSD** | 1.17 | 1.23 |

**Table 17. Precision study**

| Sr.No. | Intra Day Precession | | Inter Day Precession | |
|---|---|---|---|---|
| | **Amlodipine** | **Lisinopril** | **Amlodipine** | **Lisinopril** |
| 1 | 422654 | 741254 | 425684 | 748658 |
| 2 | 415268 | 748517 | 428898 | 754861 |
| 3 | 425786 | 721485 | 431524 | 739984 |
| 4 | 412564 | 719489 | 412584 | 732641 |
| 5 | 419856 | 715486 | 429998 | 736685 |
| 6 | 421689 | 730015 | 428733 | 740155 |
| **Average** | **419636.17** | **729374.33** | **426236.83** | **742164** |
| **SD** | **4904.827027** | **13121.17235** | **6958.716043** | **8159.43** |
| **RSD** | **1.169** | **1.799** | **1.633** | **1.099** |




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.336 | Amlodipine | 419656 | 36.522 | 1.003 | 10010 |
| 2 | 3.574 | Lisinopril | 729374 | 63.478 | 1.124 | 12102 |
| Total | | | 1149030 | 100.000 | | |

**Fig. 15. Chromatogram of precision study**

### 4.1.3 Precision

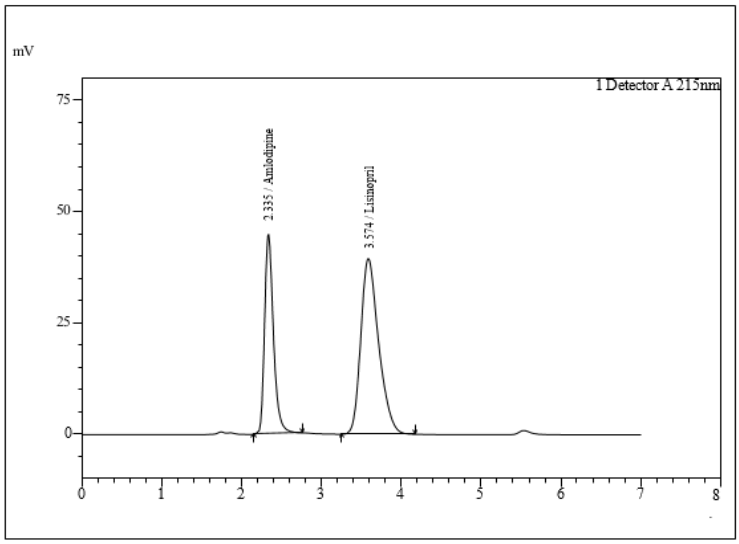
Six repetitions of the sample made from commercial tablets were injected to determine method precision, and the assay was calculated to measure the repeatability of retention periods and peak area of standard and sample. The method's precision was validated by utilising a tablet stock solution. The intraday and interday precision tests were conducted by repeating the assay six times on the same day for intraday precision and on different days for interday precision studies. The findings of this study are as follows(Table. 17):

### 4.1.4 Recovery

The approach's accuracy was determined through recovery trials at three levels (80%, 100%, and 120%) using the usual addition method. The percentage of analyte recovered was used to calculate the accuracy. The proposed method's accuracy was verified in accordance with ICH norms. For AML, a tablet powder equivalent to 5 mg AML was placed in three separate 100 ml volumetric flasks, and then 8 mg (80%), 10 mg (100%), and 12 mg (120%) of standard AML were added to each volumetric flask. The mobile phase [phosphate buffer solution: methanol (75:25 v/v)] was then poured to each volumetric flask and sonicated for 5 minutes. The solutions were then filtered, and 1 ml of the filtrate from each was placed in separate 10 ml volumetric flasks and diluted with mobile phase to the desired concentration. The solutions were injected into the chromatographic apparatus in triplicate, and the peak area was calculated to produce the percent recovery and standard deviation. The same approach was followed with Lisinopril dehydrate (Table. 18).

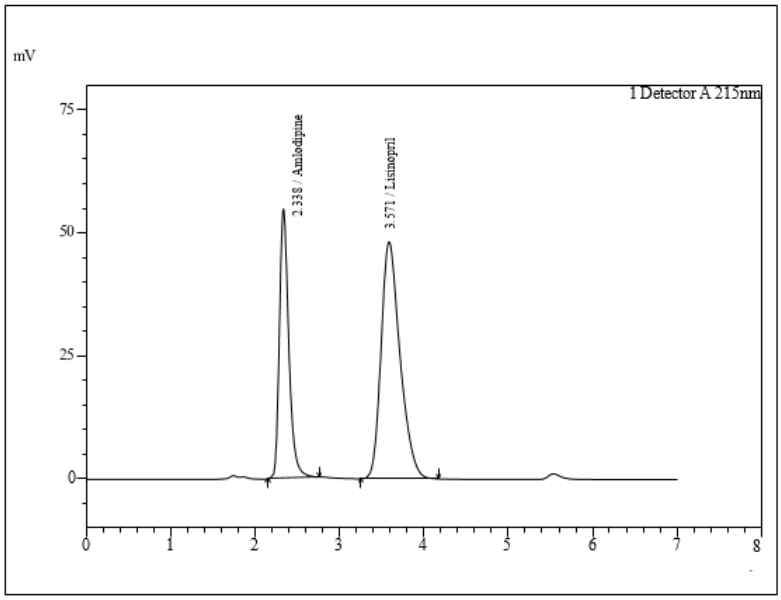
**Table 18. Recovery study**

| Drug | Label Claim | Concentration (%) | Peak Area | Concentration found | recovery% |
|---|---|---|---|---|---|
| **Amlodipine** | | 40 | 193576 | 39.7802 | 99.84 |
| | 5 | 50 | 1049181 | 50.0215 | 100.12 |
| | | 60 | 1242757 | 58.5915 | 99.29 |
| **Lisinopril** | | 40 | 361097 | 40.1548 | 100.11 |
| | 5 | 50 | 1859983 | 50.0032 | 100.03 |
| | | 60 | 2221080 | 59.9844 | 99.98 |



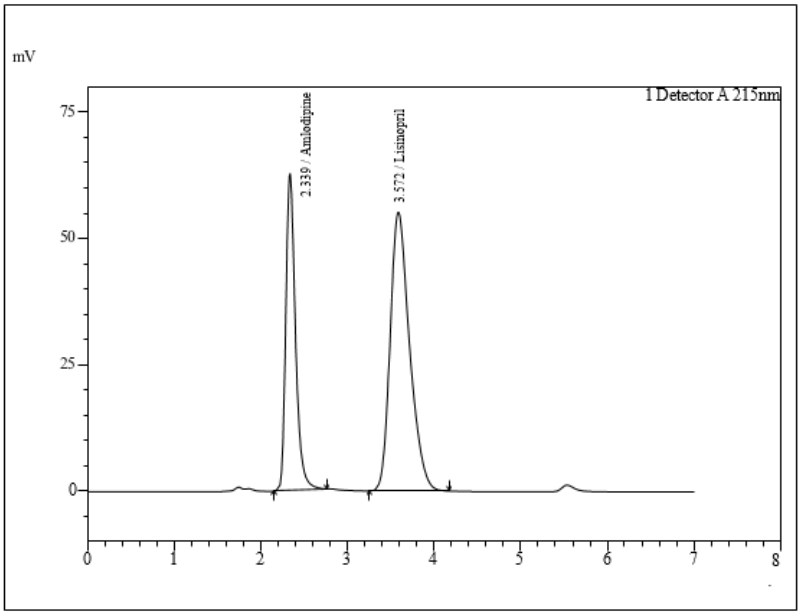

| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.335 | Amlodipine | 193576 | 34.899 | 1.007 | 9983 |
| 2 | 3.574 | Lisinopril | 361097 | 65.101 | 1.124 | 12108 |
| Total | | | 554673 | 100.000 | | |

**Fig. 16. Chromatogram of recovery study at 40ppm**




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.338 | Amlodipine | 1049181 | 36.064 | 1.011 | 9987 |
| 2 | 3.571 | Lisinopril | 1859983 | 63.936 | 1.122 | 12105 |
| Total | | | 2909164 | 100.000 | | |

**Fig. 17. Chromatogram of recovery study at 50ppm**




| Peak No. | Ret. Time | Name | Area | Area% | Tailing Factor | Theoretical Plates |
|---|---|---|---|---|---|---|
| 1 | 2.339 | Amlodipine | 1242757 | 35.878 | 1.012 | 10005 |
| 2 | 3.572 | Lisinopril | 2221080 | 64.121 | 1.123 | 12096 |
| Total | | | 3463837 | 100.000 | | |

**Fig. 18. Chromatogram of recovery study at 60ppm**

**Table 19. Robustness study**

| Sr.No. | Injection (20 µm) | Robustness for flow rate 0.9 ml % content | | Robustness for flow rate 1.1 ml % content | |
|---|---|---|---|---|---|
| | | **Amlodipine** | **Lisinopril** | **Amlodipine** | **Lisinopril** |
| 1 | 1 | 98.3 | 98.2 | 99.8 | 99.2 |
| 2 | 2 | 98.8 | 97.3 | 101.5 | 99.7 |
| 3 | 3 | 99.2 | 98.8 | 100.3 | 101.2 |
| 4 | 4 | 101.5 | 98.8 | 98.6 | 100.5 |
| 5 | 5 | 99.1 | 99.4 | 99.5 | 99.8 |
| **Average** | | **99.38** | **98.5** | **99.94** | **100.08** |
| **SD** | | **1.235718415** | **0.793725393** | **1.06911** | **0.7791** |
| **RSD** | | **1.243427666** | **0.805812582** | **1.06975** | **0.77848** |

**Table 20. LOD and LOQ Results**

| Sr.No. | Drug | LOD (%) | LOQ (%) |
|---|---|---|---|
| 1 | Amlodipine besylate | 0.024 | 0.0483 |
| 2 | Lisinopril dehydrate | 0.0027 | 0.0064 |

**Summary:**

**Table 21. Summary table**

| Parameter | Result | |
|---|---|---|
| | **Amlodipine** | **Lisinopril** |
| Calibration range (µg/ml) | 10-50 | |
| Detection wavelength (nm) | 215nm | |
| Solvent (Buffer:Methanol) | 75:25 v/v | |
| Regression equation (y*) | y = 5E-05x - 0.1239 | y = 3E-05x - 0.1259 |
| Correlation coefficient(r2) | 0.9996 | 0.9999 |
| Retention time | 2.332 ± 0.023 | 3.584 ± 0.057 |
| Area | 36.09% | 63.91% |
| Asymmetry | 1.35 | 1.30 |
| Theoretical plate | 7864 | 3005 |

#### 4.1.5 Robustness

The proposed method's robustness was tested by altering the solvent ratio in the mobile phase, flow rate, and wavelength range. The sample solutions were introduced into the chromatographic apparatus in 10 l increments. Peak area was analysed, as well as its standard deviation and percent RSD(Table. 19).

#### 4.1.6 Limit of detection and Limit of quantification (LOD, LOQ)

The suggested method's LOD and LOQ were obtained by gradually injecting lower amounts of the standard solutions under the specified chromatographic conditions. L.O.Q. = 10(SD/S) L.O.D. = 3.3(SD/S) Where SD denotes the standard deviation of the answer and S denotes the slope of the calibration curve. The slope S can be calculated using the analyte calibration curve.

## 5. CONCLUSION

With a short analytical time, the new approach provides good resolution between Amlodipine besylate and Lisinopril dehydrate. The approach is simple, accurate, fast, and precise, and it can be used for regular drug analysis without requiring any sophisticated sample preparation.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, I was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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